Carcinogenesis vol.20 no.7 pp.1375–1378, 1999

SHORT COMMUNICATION

Chemoprevention of tobacco smoke-induced lung tumors in A/J strain mice with dietary *myo*-inositol and dexamethasone

Hanspeter Witschi[1], Imelda Espiritu and Dale Uyeminami

Institute of Toxicology and Environmental Health and Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, CA 95616, USA

[1]To whom correspondence should be adressed at: ITEH, University of California, 1 Shields Avenue, Davis, CA 95616, USA
Email: hrwitschi@ucdavis.edu

Male A/J strain mice were fed AIN-76A diet supplemented with *myo*-inositol/dexamethasone (10 g and 0.5 mg/kg diet) or acetylsalicylic acid (300 mg/kg) and exposed for 5 months to a mixture of sidestream and mainstream cigarette smoke at a concentration of 132 mg total suspended particulates/m³. After tobacco smoke exposure, they were allowed to recover for another 4 months in filtered air. In the animals fed AIN-75A diet alone or acetylsalicylic acid, the average number of tumors/lung was 2.1, whereas in the animals given the *myo*-inositol/dexamethasone diet, the average lung tumor multiplicity was 1.0 ($P < 0.05$). In animals exposed to filtered air, lung tumor multiplicities were 0.6 for animals fed AIN-76A or *myo*-inositol/dexamethasone and 1.2 for animals fed acetylsalicylic acid. It was concluded that the combination of *myo*-inositol and dexamethasone constitutes an effective chemopreventive regimen against tobacco smoke-induced lung tumorigenesis.

Identification of chemicals that might prove useful in the prevention of lung cancer, particularly tobacco smoke-induced lung cancer, continues to be an area of intense research (1,2). Most putative chemopreventive agents are evaluated in animal models of lung tumorigenesis. During the last few years, the strain A mouse lung tumor assay has become the preferred tool for the evaluation of the effects of a large variety of agents. They include isothiocyanates (3), selenium compounds (4), steroid hormones (5), polyphenols (6) and non-steroidal anti-inflammatory agents (7). Lung tumors are usually produced by specific chemical carcinogens that are thought to play a crucial role in tobacco carcinogenesis, such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) or benzo[*a*]pyrene (B[a]P). A test agent is judged to be a potentially useful chemopreventive agent if treatment of the animals before, during or after carcinogen exposure significantly reduces the number of lung tumors (6). However, no animal model of tobacco smoke carcinogenesis has been used in such screening experiments. One reason might be that it is difficult to produce tobacco smoke-induced lung tumors in laboratory animals with high incidence, even in long-term experiments involving exposure to high levels of tobacco smoke (8,9). The lack of screening experiments involving tobacco smoke may have been partially responsible for some unanticipated results in clinical trials, such as the recent observation that β-carotene, rather than preventing lung cancer development, actually has the opposite effect (10). An animal model for tobacco smoke carcinogenesis has now become available. We have conducted several experiments in which we found that exposure of strain A/J mice to a mixture of sidestream and mainstream cigarette smoke can significantly increase lung tumor multiplicity and incidence (11). The effects of three chemopreventive agents have been evaluated in this animal model: phenethylisothiocyanate (PEITC), *N*-acetylcysteine (NAC) and green tea (12). While these three agents are highly successful in preventing the development of lung tumors following exposure to NNK (13–15), NAC and green tea had no chemopreventive action against tobacco smoke and the effect of PEITC was marginal, but not significant. In the present investigation we examined three additional chemicals that have been found to have chemopreventive action against NNK-induced lung tumors: a mixture of *myo*-inositol and dexamethasone (16) and the common drug aspirin (acetylsalicylic acid) (17).

Male A/J strain mice were purchased from Jackson Laboratories (Bar Harbor, ME). A standard rodent health surveillance screen showed no evidence for infectious or parasitic disease. The animals were kept within inhalation chambers in polypropylene cages with tightly fitting wire screen lids on conventional bedding material and had at all times during the experiment, including during smoke exposure, access *ad libitum* to water and the test diets. The animals were observed daily and weighed weekly. The inhalation chambers were kept on a 12 h light/dark cycle and controlled for temperature and humidity. Kentucky 1R4F reference cigarettes were from the same source as described previously (18). Acetylsalicylic acid, *myo*-inositol dexamethasone and corn oil were obtained from Sigma Chemical Co. (St Louis, MO). The AIN-76A test diet (20% casein, 0.3% DL-methionine, 15% corn starch, 55% sucrose, 5% cellulose, 3.5% mineral mix, 1% vitamin mix and 0.2% choline bitartrate) was purchased in powdered form from Dyets (Bethlehem, PA). Diets containing acetylsalicylic acid or *myo*-inositol/dexamethasone were prepared freshly every other week by adding the appropriate amounts of the ingredients plus 50 ml corn oil/kg diet and by mixing thoroughly in a Hobart blender. Final concentrations were 300 mg/kg acetylsalicylic acid (17) and 10 g/kg and 0.5 mg/kg for the *myo*-inositol/dexamethasone diet (16). All diets were stored at 4°C.

In order to confirm the effectiveness of *myo*-inositol/dexamethasone and of acetylsalicylic acid, as reported by others (16,17), a positive control experiment was done concomitant with the main experiment. Three groups of mice were placed on the AIN-76A, *myo*-inositol/dexamethasone or acetylsalicylic acid diets. Two weeks later, all animals received four i.p. injections of NNK at a dose of 50 mg/kg at weekly intervals. Feeding of the three diets then continued until 4 months after the last NNK injection, when the animals were killed and lung tumors counted.

In the main experiment, the 8–10-weeks-old A/J strain mice

Abbreviations: B[a]P, benzo[*a*]pyrene; NAC, *N*-acetylcysteine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; PEITC, phenethylisothiocyanate; TSP, total suspended particulates.

© Oxford University Press

This article is for individual use only and may not be further reproduced or stored electronically without written permission from the copyright holder.
Unauthorized reproduction may result in financial and other penalities, (c) OXFORD UNIV PRESS

PM3000457022

Source: https://www.industrydocuments.ucsf.edu/docs/kmcl0001

Table I. Exposure data[a]

Parameter	Weeks 1 and 2	Weeks 3–5	Weeks 6–22
Temperature (°C)	21 ± 3 (9)	25 ± 1 (11)	24 ± 1 (90)
Relative humidity (%)	33 ± 11 (9)	34 ± 8 (11)	39 ± 8 (90)
Total suspended particulates (TSP) (mg/m^3)	71 ± 8 (18)	86 ± 12 (22)	132 ± 18 (72)
Nicotine (mg/m^3)	NS[b]	NS[b]	26.2 ± 9.3 (40)

[a]All measurements from within the inhalation chambers. Data are given as means ± SD; number of determinations in parentheses.
[b]No samples available.

were assigned at random to the following groups: group 1, fed control diet AIN-76A and exposed to tobacco smoke (1A) or kept in filtered air (1B); group 2, fed *myo*-inositol/dexamethasone and exposed to tobacco smoke (2A) or filtered air (2B); group 3, fed acetylsalicylic acid and exposed to tobacco smoke (3A) or kept in filtered air (3B). The animals were placed, within their cages, in stainless steel inhalation chambers ventilated with tobacco smoke or filtered air. Exposure to tobacco smoke was for 6 h/day, 5 days/week, during the first 2 weeks to an average of 71 mg total suspended particulates (TSP)/m^3, then for another 3 weeks to 86 mg/m^3 and finally for the remainder of the experiment to an average of 132 mg/m^3. All cages in the exposure chambers were rotated periodically so that each individual cage occupied at least once all possible locations in each inhalation chamber (18). The tobacco smoke exposure system and analytical methods used to measure nicotine, CO and TSPs were identical to the ones described in detail previously (18,19). Exposure conditions are listed in Table I. After 5 months, all mice were removed from the inhalation chambers and transferred to a conventional animal holding facility with controlled temperature and humidity (20–21°C, 40–70% humidity, 12 h light/dark cycle). Feeding of the test diets continued during the recovery period. Nine months after the beginning of the experiment, the animals were killed and the lungs prepared for tumor analysis as described previously (12,18).

After fixation of the lungs in Tellyesniczky's fluid, the number of tumor nodules visible on the lung surface was counted and their largest diameter was measured. The results were expressed as tumor incidence, i.e. percentage of animals with one or several lung tumors/total animals at risk, and as tumor multiplicity, calculated as average number of tumors/lung of all animals, including non-tumor-bearing ones. Data on lung tumor multiplicity were analyzed by parametric ANOVA followed by the Tukey–Kramer multiple comparisons test and by non-parametric ANOVA with Dunn's post-test. Lung tumor incidences were compared using Fisher's exact test. A P value of ≤ 0.05 was considered to be significant.

The results of the positive control experiment, carried out concomitantly with the main experiment and using mice received in the same shipment, are shown in Table II. When all lung tumors were counted, it was found that animals fed the *myo*-inositol/dexamethasone diet had significantly fewer tumors/lung than did animals fed the AIN-76A diet. On the other hand, acetylsalicylic acid had no effect. According to the procedure suggested by Duperron and Castonguay (17), lung tumor multiplicities were also calculated by counting only tumors with a diameter of ≥1 mm. It was found that acetylsalicylic acid produced significantly fewer tumors of this

Table II. Positive control data

Treatment[a]	Lung tumor multiplicity[b]	
	All tumors	Tumors ≥1 mm diameter
AIN-76A + NNK	15.1 ± 1.4 (21/22)	8.2 ± 0.9 (20/22)
Myo + NNK	2.1 ± 0.5 (12/15)[c]	0 (0/15)[c]
ASA + NNK	16.0 ± 1.4 (20/20)	5.7 ± 0.8 (19/20)[c]

Myo, *myo*-inositol/dexamethasone diet (10 g and 0.5 mg/kg diet); ASA, acetylsalicylic acid diet (300 mg/kg diet).
[a]All animals were placed on the diet and, beginning 2 weeks later, given four weekly injections of 50 mg/kg NNK; animals were killed 4 months after the last injection.
[b]Data given as means ± SE. The number of tumor-bearing animals/total number of animals in the group are given in parentheses
[c]Significantly different from animals fed AIN-76 alone.

size within the 5 month experimental period than in the controls. *myo*-inositol/dexamethasone completely prevented growth of tumors to a size of ≥1 mm (Table 2).

In the main experiment, the animals exposed to tobacco smoke were gradually acclimatized as shown in Table 1. During the first 2 weeks, the animals were exposed to an average TSP concentration of 71 mg/m^3, which was then increased during weeks 3–5 to 86 mg/m^3, a concentration similar to that used in previous studies (11,12,18). From week 6 until the end of the experiment, TSP concentrations were maintained at ~132 mg/m^3. No obvious signs of toxicity were noticed, although, as in previous studies, we again observed some early deaths of animals (within the first 6 weeks): in the mice exposed to tobacco smoke, two animals fed AIN-76A (1A), three animals fed *myo*-inositol/dexamethasone (2A) and two animals fed acetylsalicylic acid (3A). In animals kept in filtered air, two in the *myo*-inositol-dexamethasone group (2B) and three in the acetylsalicylic acid group (3B) died. No animal fed diet AIN-76A alone (1B) died.

Weight gains by the mice during the experiment are shown in Table III. While the animals were exposed to tobacco smoke, they gained weight at a slower rate than did the animals kept in filtered air. After 5 months they had reached only ~85% of the body weight found in controls. After removal from the smoke, they gained weight somewhat faster than did the animals kept in filtered air at all times and 2 months later had approximately the same body weights.

Lung tumor counts in the animals exposed to tobacco smoke and fed the different diets are shown in Table IV(A). In animals fed the control diet, an average of 2.1 lung tumors were found when all tumors were counted; for tumors ≥1 mm in diameter, the average lung tumor multiplicity was 1.7. *myo*-inositol/dexamethasone significantly reduced tumor multiplicity to 1.0 or 0.7, respectively, ~40–45% of values found in the tobacco smoke/AIN-76A animals. In addition, *myo*-inositol/dexamethasone also produced a significant reduction in lung tumor incidence. On the other hand, acetylsalicylic acid had no effect when all lung tumors were counted and only a small, statistically insignificant effect when tumors measuring ≥1 mm were counted. Lung tumor incidence was not affected.

In the animals exposed to filtered air, lung tumor multiplicities were significantly lower throughout than they were in animals exposed to tobacco smoke and fed either AIN-76A or acetylsalicylic acid [Table IV(B)]. The latter actually appeared to double lung tumor multiplicity in the filtered air group, compared with the animals fed AIN-76A or *myo*-inositol/

PM3000457023

Source: https://www.industrydocuments.ucsf.edu/docs/kmcl0001

Table III. Body weights (g)[a]

Time[b]	Group 1A	Group 1B	Group 2A	Group 2B	Group 3A	Group 3B
0	23 ± 1	24 ± 2	24 ± 2	24 ± 2	24 ± 2	24 ± 2
1 month	23 ± 2	28 ± 2	22 ± 2	27 ± 3	23 ± 2	28 ± 2
2 months	24 ± 2	29 ± 3	23 ± 3	28 ± 3	24 ± 1	28 ± 3
3 months	25 ± 3	30 ± 3	25 ± 3	30 ± 4	25 ± 2	30 ± 3
4 months	26 ± 3	30 ± 3	25 ± 4	31 ± 4	26 ± 3	31 ± 3
5 months	27 ± 3	31 ± 3	26 ± 1	32 ± 5	27 ± 2	31 ± 4
6 months	30 ± 4	31 ± 4	29 ± 4	33 ± 5	30 ± 3	31 ± 4
7 months	32 ± 4	33 ± 3	31 ± 3	33 ± 5	32 ± 4	32 ± 4
8 months	34 ± 4	34 ± 3	32 ± 6	34 ± 6	35 ± 5	32 ± 5
9 months	35 ± 5	35 ± 3	32 ± 6	34 ± 6	36 ± 6	32 ± 6
	(35)	(30)	(34)	(25)	(35)	(25)

[a]All data given as means ± SD. Group 1, fed AIN-76A diet; group 2, *myo*-inositol/dexamethasone (10 g/kg and 0.5 mg/kg diet); group 3, acetylsalicylic acid (300 mg/kg diet).
[b]Animals in groups 1A, 2A and 3A were exposed for 5 months to tobacco smoke and then removed to filtered air; animals in groups 1B, 2B and 3B were kept in filtered air throughout. All animals were killed 9 months after the beginning of the experiment.

Table IV. Lung tumor data

Treatment[a]	All tumors		Tumors ≥1 mm diameter	
	Multiplicity[b]	Incidence[c]	Multiplicity[b]	Incidence[c]
(A) Tobacco smoke-exposed animals[d]				
AIN-76A (1A)	2.1 ± 0.3 (35)	30/35 (85%)	1.7 ± 0.2 (35)	28/35 (80%)
Myo (2A)	1.0 ± 0.2 (34)[e]	21/34 (62%)[f]	0.7 ± 0.1 (34)[e]	18/34 (53%)[f]
ASA (3A)	2.2 ± 0.2 (35)	32/35 (91%)	1.3 ± 0.2 (35)	28/35 (80%)
(B) Animals kept in filtered air[g]				
AIN-76A (1B)	0.6 ± 0.1 (30)	15/30 (50%)	0.4 ± 0.1 (30)	10/30 (33%)
Myo (2B)	0.6 ± 0.2 (25)	11/25 (44%)	0.2 ± 0.1 (25)	6/25 (24%)
ASA (3B)	1.2 ± 0.2 (25)	19/25 (76%)	0.8 ± 0.1 (25)	16/26 (61%)

[a]Myo, *myo*-inositol/dexamethasone diet (10 g/kg and 0.5 mg/kg diet); ASA, acetylsalicylic acid diet (300 mg/kg diet).
[b]Average number of tumors per lung, including non-tumor-bearing animals. Data are given as means ± SD with the number of animals in parentheses.
[c]Number of tumor-bearing animals/total number of animals at risk.
[d]Animals exposed to tobacco smoke for 5 months, followed by a 4 month recovery period in filtered air.
[e]Significantly different ($P < 0.05$) from animals fed AIN-76A or ASA with parametric or non-parametric ANOVA followed by the appropriate post-test.
[f]Significantly different ($P < 0.05$) from animals fed AIN-76A or ASA with Fisher's exact test.
[g]Values for groups 1B and 3B are significantly lower than for animals exposed to tobacco smoke in groups 1A and 3A.

dexamethasone, but the difference was not statistically significant when data were analyzed by parametric or non-parametric ANOVA. Lung tumor incidences were throughout lower compared with animals exposed to tobacco smoke.

In previous experiments we found that the average lung tumor multiplicity in strain A mice exposed to a mixture of sidestream and mainstream cigarette smoke was ~1.3–1.4 tumors/lung (11). The same tumor yield was obtained in mice exposed to full smoke (18) or exposed to the gas phase only (20). In the present investigation, we increased tobacco smoke concentrations, measured as TSP, from an average of 80–90 mg/m^3 to an average of 130 mg/m^3. As a result, tumor yield increased from an average of 1.3 tumors/lung to an average of 2 tumors/lung, while control values stayed within the range 0.5–0.8 tumors/lung that is usually found in strain A mice of this age (21).

The diet containing *myo*-inositol and the synthetic glucocorticoid dexamethasone proved to be a highly effective chemopreventive regimen. A significant reduction in both lung tumor multiplicity and in lung tumor incidence between animals exposed to tobacco smoke and AIN-76 or *myo*-inositol/dexamethasone diet was observed. The number of tumors/lung in the animals fed the *myo*-inositol/dexamethasone mixture and exposed to tobacco smoke actually approached the tumor multiplicity in animals exposed to filtered air. A significant reduction in lung tumor formation caused by administration of *myo*-inositol, dexamethasone or a mixture of the two compounds has been demonstrated previously in strain A mice treated with B[a]P and with NNK (5,16). Our data suggest that a combination of the two compounds is also highly effective against the carcinogenic effects of the complex mixture of full tobacco smoke.

The other agent we tested, acetylsalicylic acid, has been thoroughly evaluated by Castonguay and associates, together with some other non-steroidal anti-inflammatory agents (17,22,23). These might be particularly promising chemopreventive agents because they are approved human drugs and there is evidence suggesting that they might prevent development of colorectal cancer in man. (24). In strain A mice treated with repeated doses of NNK, acetylsalicylic acid greatly reduced the development of lung tumors with a diameter >1 mm, suggesting that it might effectively slow tumor growth (17). In the present study, we could duplicate this finding in our positive control experiment, although we administered NNK as four injections rather than continuously in the drinking water. However, when all tumors were counted, no protective effect was seen. In the main experiment a small and statistically insignificant reduction in tumor formation was only seen when tumors >1 mm were counted.

PM3000457024

Source: https://www.industrydocuments.ucsf.edu/docs/kmcl0001

From the present experiments we conclude that addition to the diet of *myo*-inositol and dexamethasone is the most promising chemopreventive treatment among the six agents (PEITC, NAC, green tea, *myo*-inositol, dexamethasone and acetylsalicylic acid) that so far have been investigated in an animal model where lung tumors are induced by tobacco smoke rather than by selected model chemicals. This raises the question whether it is preferable to screen for chemopreventive agents in animals treated with model lung carcinogens or in animals exposed to full smoke? Studies with NNK and B[a]P are certainly more expedient and give higher tumor numbers in a shorter time than do inhalation studies. On the other hand, it should be remembered that nitrosamines and polycyclic hydrocarbons are not necessarily representative of all putative carcinogens that occur in tobacco smoke (20). Inhalation studies thus appear to be more realistic.

The question may be raised what role chemopreventive agents should play in the fight against lung cancer? It was pointed out that, although chemoprevention should never substitute for quitting smoking, there might be several populations possibly benefitting from chemoprevention, particularly former smokers, where chemoprevention could produce an additional reduction in risk (3). Previous observations suggest that a combination of *myo*-inositol and dexamethasone is also effective in strain A mice if applied after carcinogen exposure (16). This will make it important to evaluate the effects of these two compounds in A/J strain mice after they have been exposed to tobacco smoke.

Acknowledgements

This publication was made possible by grants numbers ES07908 and ES05707 from the National Institute of Environmental Health Sciences (NIEHS). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIEHS, NIH.

References

1. Benner,S.E., Lippman,S.M. and Hong,W.K. (1995) Chemoprevention of lung cancer. *Chest*, **107**, 316S–321S.
2. Karp,D.D. (1997) Lung cancer chemoprevention and management of carcinoma *in situ*. *Semin. Oncol.*, **24**, 402–410.
3. Hecht,S.S. (1996) Chemoprevention of lung cancer by isothiocyanates. *Adv. Exp. Med. Biol.*, **401**, 1–11.
4. el-Bayoumy,K., Upadhyaya,P., Chae,Y.H., Sohn,O.S., Rao,C.V., Fiala,E. and Reddy,B.S. (1995) Chemoprevention of cancer by organoselenium compounds. *J. Cell. Biochem.*, **22** (suppl.), 92–100.
5. Estensen,R.D. and Wattenberg,L.W. (1993) Studies of chemopreventive effects of myo-inositol on benzo[*a*]pyrene-induced neoplasia of the lung and forestomach of female A/J mice. *Carcinogenesis*, **14**, 1975–1977.
6. Stoner,G.D. and Mukhtar,H. (1995) Polyphenols as cancer chemopreventive agents. *J. Cell. Biochem.*, **22** (suppl.), 169–180.
7. Castonguay,A., Rioux,N., Duperron,C. and Jalbert,G. (1998) Inhibition of lung tumorigenesis by NSAIDS: a working hypothesis. *Exp. Lung Res.*, **24**, 605–615.
8. Mohr,U. and Reznik G. (1978) Tobacco carcinogenesis. In Harris,C.C. (ed.) *Pathogenesis and Therapy of Lung Cancer*. Marcel Dekker, New York, NY, pp. 263–368.
9. Coggins,C.R. (1998) A review of chronic inhalation studies with mainstream cigarette smoke in rats and mice. *Toxicol. Pathol.*, **26**, 307–314.
10. De Luca,L.M. and Ross,S.A. (1996) Beta-carotene increases lung cancer incidence in cigarette smokers. *Nutr. Rev.*, **54**, 178–180.
11. Witschi,H. (1998) Tobacco smoke as a mouse lung carcinogen. *Exp. Lung Res.*, **24**, 385–394.
12. Witschi,H.P., Espiritu,I., Yu,M. and Willits,N.H (1998) The effects of phenethyl isothiocyanate (PEITC), *N*-acetylcysteine (NAC) and green tea on tobacco smoke-induced lung tumors in strain A/J mice. *Carcinogenesis*, **19**, 1789–1784.
13. Hecht,S.S. (1997) Approaches to chemoprevention of lung cancer based on carcinogens in tobacco smoke. *Environ. Health Perspect.*, **105** (suppl. 4), 955–963.
14. De Flora,S., Cesarone,C.F., Balansky,R.M., Albini,A., D'Agostini,F., Bennicelli,C., Bagnasco,M., Camoirano,A., Scatolini,L. and Rovida,A. (1995) Chemopreventive properties and mechanisms of *N*-acetylcysteine. The experimental background. *J. Cell. Biochem.*, **22** (suppl.), 33–41.
15. Cao,J., Xu,Y., Chen,J. and Klaunig,J.E. (1996) Chemopreventive effects of green and black tea on pulmonary and hepatic carcinogenesis. *Fundam. Appl. Toxicol.*, **29**, 244–250.
16. Wattenberg,L.W. and Estensen,R.D. (1996) Chemopreventive effects of *myo*-inositol and dexamethasone on benzo[*a*]pyrene and 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone-induced pulmonary carcinogenesis in female A/J mice. *Cancer Res.*, **56**, 5132–5135.
17. Duperron,C. and Castonguay,A. (1997) Chemopreventive efficacies of aspirin and sulindac against lung tumorigenesis in A/J mice. *Carcinogenesis*, **18**, 1001–1006.
18. Witschi,H., Espiritu,I., Peake,J.L., Wu,K., Maronpot,R.R. and Pinkerton,K.E. (1997) The carcinogenicity of environmental tobacco smoke. *Carcinogenesis*, **18**, 575–586.
19. Teague,S.V., Pinkerton,K.E., Goldsmith,M., Gebremichael,A.C.S., Jenkins,R.A. and Moneyhun,J.H. (1994) Sidestream cigarette smoke generation and exposure system for environmental tobacco smoke studies. *Inhal. Toxicol.*, **6**, 79–93.
20. Witschi,H., Espiritu,I., Maronpot,R.R., Pinkerton,K.E. and Jones,A.D. (1997) The carcinogenic potential of the gas phase of environmental tobacco smoke. *Carcinogenesis*, **18**, 2035–2042.
21. Stoner,G.D. and Shimkin,M.B. (1985) Lung tumors in strain A mice as a bioassay for carcinogenicity. In Milman,H. and Weisburger,E.K. (eds) *Handbook of Carcinogen Testing*. Noyes Publications, Park ridge, NJ, pp. 179–184.
22. Castonguay,A. and Rioux,N. (1997) Inhibition of lung tumourigenesis by sulindac: comparison of two experimental protocols. *Carcinogenesis*, **18**, 491–496.
23. Malkinson,A.M., Koski,K.M., Dwyer-Nield,L.D., Rice,P.L., Rioux,N., Castonguay,A., Ahnen,D.J., Thompson,H., Pamukcu,R. and Piazza,G.A. (1998) Inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced mouse lung tumor formation by FGN-1 (sulindac sulfone). *Carcinogenesis*, **19**, 1353–1356.
24. Trujillo,M.A., Garewal,H.S. and Sampliner,R.E. (1994) Nonsteroidal antiinflammatory agents in chemoprevention of colorectal cancer. At what cost? *Dig. Dis. Sci.*, **39**, 2260–2266.

Received December 29, 1998; revised February 19, 1999; accepted March 17, 1999

PM3000457025

Source: https://www.industrydocuments.ucsf.edu/docs/kmcl0001